

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) EP 0 982 587 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
01.03.2000 Bulletin 2000/09

(51) Int Cl⁷: G01N 30/60

(21) Application number: 99306500.2

(22) Date of filing: 18.08.1999

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 21.08.1998 US 138434

(71) Applicant: BIO-RAD LABORATORIES, INC.
Hercules California 94547 (US)

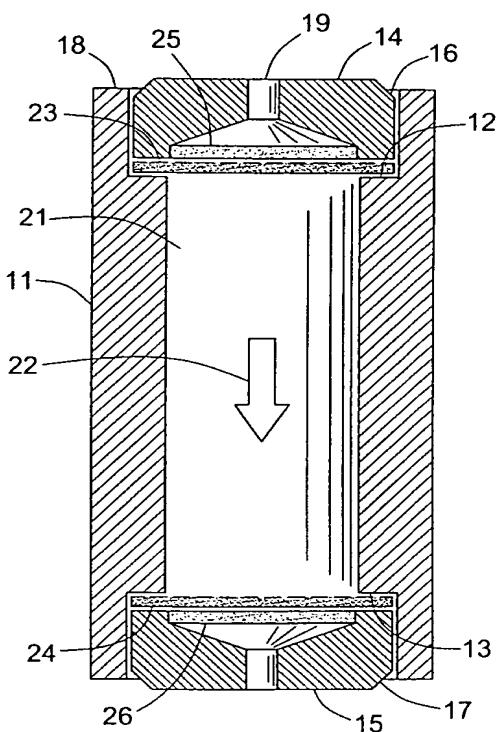
(72) Inventor: Dewaele, Chris
9810 Nazareth (BE)

(74) Representative: O'Brien, Caroline Jane et al
MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP (GB)

(54) Chromatography column cartridge having filter with graded porosity

(57) Cartridges (11) for high performance liquid chromatography are designed for use with a large number of repeated injections without excessive buildup of back pressure by the inclusion of a filter medium (23,24) consisting of sintered stainless steel fibers formed into a nonwoven structure whose pore size decreases in the direction of flow down to a pore size of less than ten microns.

Fig. 1



Description**BACKGROUND OF THE INVENTION**

[0001] This invention relates to column chromatography, and in particular to columns designed to separate components of whole blood.

[0002] Chromatography in general and high-performance liquid chromatography (HPLC) in particular are widely used for analytical and preparative separations of mixtures of chemical species. The columns used for HPLC are typically used for a series of successive analyses, particularly in automated chromatographic systems, and in general where the repeated use of a single column permits direct sample-to-sample comparisons without the error that is introduced by cleaning the separatory resin between injections or by exchanging one column for a fresh column of the same composition. Solid debris often enters these columns, having been entrained by the sample or the carrier fluid, and accumulates in the column, obstructing the free flow of fluid and causing back pressure to rise. The debris arises from several sources. Whole blood, for example, either diluted or undiluted, contains cell wall fragments and other insoluble matter. Included among this insoluble matter are insoluble proteins as well as soluble proteins that agglomerate and/or precipitate. Another source is the chromatographic instrument itself. Components of the instrument such as valves and pump seals tend to disintegrate and release particulate matter into the carrier fluid.

[0003] Debris from all of these sources tend to clog the column, inhibiting the free flow of liquid through the column and causing the back pressure to rise. Metal frits are usually present in the column to hold the resin, and these frits act as filters for the solid debris, but the frits themselves become clogged with the debris, again causing the column back pressure to rise. One of the sites where flow blockage occurs is the interface between the frit and the resin, since the contact between the frit and the resin reduces the sizes of the interstitial spaces in the interfacial region, rendering them narrower than those of either the frit or the resin themselves. The spaces at the interfacial region are small enough to block even the passage of very small proteinaceous material. This not only causes back pressure to rise but also interferes with the separation when these proteins are among the components sought to be identified and/or quantified.

[0004] Some of the debris can be removed by filtration or centrifugation that is off-line (*i.e.*, before injection into any continuous-flow column system). This is a time-consuming step, however, and provides an added source of operator error. For on-line systems (*i.e.*, automated, continuous-flow systems), guard columns and prefilters that are not in direct contact with the resin have been included, but for the reasons set forth in the preceding paragraph, back pressure still rises with repeated injec-

tions. The rising back pressure typically requires that the guard columns be replaced after 50 to 200 injections, depending on the application.

5 SUMMARY OF THE INVENTION

[0005] A highly effective filter medium has now been discovered for removing debris present in chromatographic samples and carrier fluids. The filter medium removes the debris and yet permits the passage of many more samples than filters of the prior art. The filter medium is a multi-layered structure of non-woven sintered fibers of stainless steel, the fibers being progressively more densely packed in the direction of flow. This results in progressively decreasing pore sizes which are the interstitial spaces between the fibers. The decrease in interstitial clearance may be attributable to a greater number of fibers per unit volume or to the use of fibers of a progressively smaller diameter. Most conveniently, however, fibers of a single diameter are used, and the variation is created by stepwise changes in the packing density of the fibers. In any event, the interstitial clearance reduces to a very small diameter sufficient to retain the debris associated with whole blood and yet the filter medium has a greatly reduced tendency to clog.

[0006] A surprising effect of the use of a filter medium of this description is that it can be placed in direct contact with a separatory resin without creating an interfacial region of reduced pore size. This is of particular value in the analysis of samples of whole blood, since it allows the blood proteins of small size to pass through from the filter medium to the resin without causing an increased blockage at the interface between the filter medium and the resin. In addition to whole blood samples, however, the present invention is applicable to analytical and preparative samples in general that have a tendency to cause a gradual increase in back pressure in a chromatographic column after repeated injections through the column.

40 BRIEF DESCRIPTION OF THE FIGURE

[0007] The figure attached hereto is an exploded view in cross section of an HPLC cartridge incorporating a filter medium in accordance with the present invention.

45 DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[0008] The gradation of the pore size is oriented so that the debris-containing sample fluid enters the filter through the side with the larger interstitial spaces, passes through the filter, and leaves the filter through the side with the smaller interstitial spaces. The degree and spatial rate of gradation of size may vary within the scope of this invention, as may the pore sizes themselves. Best results however will be obtained with a pore size gradation that decreases to an ultimate size (on the down-

stream side of the filter) that is narrow enough to retain particles that are 10 microns in diameter, preferably 5 microns in diameter, and most preferably 1 to 3 microns in diameter.

[0009] Other relevant factors of the filter are its thickness and permeability factor, both of which may range as may the pore size. The thickness in most cases will be within the range of from about 0.1 mm to about 1.0 mm, preferably from about 0.2 mm to about 0.6 mm, and most preferably from about 0.3 mm to about 0.6 mm. The permeability factor k is defined by Darcy's Law:

$$\frac{\Delta P}{L} = \frac{\mu}{k} V$$

where ΔP is the pressure drop, L is the thickness of the filter, μ is the fluid viscosity, and V is the superficial velocity. The permeability factor in most cases will be within the range of from about 4×10^{-13} to about 1×10^{-10} m², preferably from about 4×10^{-13} to about 1×10^{-11} m², and most preferably from about 4×10^{-13} to about 4×10^{-12} m².

[0010] The gradation may be stepwise or continuous, and the variation from the larger-pore side to the small-pore side may vary widely. The pore size differential (*i.e.*, the difference between the largest pore size and the smallest pore size) may thus range from about 1 micron to about 50 microns, or preferably from about 2 microns to about 20 microns. In filter media will stepwise gradations, the filter will contain two or more gradations, preferably three to six, and the pore size on the coarse (upstream) side of the filter (the coarsest portion of the filter) may range from about 2 microns to about 50 microns, preferably from about 2 microns to about 6 microns.

[0011] The filter material is sintered non-woven stainless steel fibers. The particular stainless steel is not critical to the invention, and a variety of different stainless steel alloys can be used. Austenitic stainless steels, *i.e.*, those whose chief alloying elements are chromium and nickel, are preferred. A particularly preferred stainless steel is 316L stainless steel, whose composition is approximately 0.03 % carbon, 2.00% manganese, 1.00% silicon, 16.0-18.0% chromium, 10.0-14.0% nickel, 0.45% phosphorus, 0.03% sulfur, and 2.0-3.0% molybdenum (all percents by weight). Examples of other useful stainless steels are 304, 304H, 304L, 304 LN, 316, 316F, 316H, 316LN, 316N, 317, 317L, 321, 321H, 347, 347H, 348, 348H, and 384.

[0012] A currently preferred filter medium that meets the parameters of this invention is BEKIPOR® ST filter medium, and in particular BEKIPOR® ST 3AL3, a product of NV Bekaert SA of Belgium, available through Bekaert Fibre Technologies Europe, Zwevegem, Belgium, and Bekaert Corporation, Atlanta, Georgia, USA. This medium is made of 316L stainless steel fibers, randomly compressed in a non-woven structure and sintered, and is supplied in sheets, with typical lateral dimensions of 1180 mm × 1500 mm and 0.35 mm in thick-

ness. This particular product has an absolute filter rating of 3 microns, a bubble point pressure of 12,300 Pa (ASTM E 128061, equivalent ISO 4003), an average air permeability of 9 L/dm²/min at 200 Pa (NF A 95-352, equivalent IOS 4022), a permeability factor k of 4.80 × 10⁻¹³, a weight of 975 g/m², a porosity of 65%, and a dirt holding capacity of 6.40 mg/cm² according to Multipass method ISO 4572 with 8" initial differential pressure. Other media of similar characteristics and made of similar materials can also be used.

[0013] In accordance with this invention, the filter media are incorporated into cartridges used for HPLC or other forms of chromatography. An example of such a cartridge is shown in a longitudinal exploded cross section in the attached drawing. The cartridge consists of a hollow cylindrical tube 11, generally of metal such as stainless steel, and open at each end. The interior of each end of the tube has a shoulder 12, 13 extending around the circumference of the inner tube surface. End plugs 14, 15 fit in each end of the tube, resting on the shoulders. Each end plug terminates in a frustum 16, 17, so that the plug can be sealed into the end of the tube by a rolling tool which curves the end edge 18 of the tube inward over the frustum, compressing the end plug against the shoulder to seal the end plug in place and seal the end of the tube closed except for a narrow orifice 19 in the end plug that permits passage of the sample. The interior portion 21 of the cylindrical tube between the shoulders is a chamber for retaining the column packing used as a chromatographic separation medium, and the intended direction of flow of the sample and carrier fluid through the cartridge is indicated by the arrow 22.

[0014] Bordering the separation medium chamber 21 at both the upstream and downstream ends are disks 23, 24 of the sintered non-woven stainless steel fiber filter medium described above. While this particular embodiment contains such a disk at each end of the chamber, the cartridge may also contain a single such disk at the inlet (upstream) end only. In either case, in view of the gradation of pore size in each filter medium, the flow direction is significant, and the disks are oriented such that the side with the larger pore size is at the upstream side relative to the flow direction. As sheets of the filter medium are supplied by commercial suppliers of the medium, the side with the larger pore size is frequently differentiated by being colored or otherwise marked to guide the user in obtaining the desired orientation. In the drawing, the side with the larger pore size is indicated by a dashed line on each filter medium disk. Since the filter medium is thin and flexible, mechanical support is preferably provided to assure that the filter medium remains flat. The mechanical support can be provided by another porous sintered stainless steel disk 25, 26, a perforated metal plate, or an equivalent structure. Only the filter medium itself 23, 24, however, contacts the resin.

[0015] Once assembled, the cartridge is mounted into

a cartridge holder in a chromatography apparatus. Such holders are widely used in on-line chromatography systems, are well known to those skilled in the art, and are readily available from suppliers of HPLC equipment. Examples of suitable cartridge holders are those described in McEachern, U.S. Patent No. 4,563,275, issued January 7, 1986, and Brownlee, U.S. Patent No. 4,283,280, issued August 11, 1981, both of which are incorporated herein by reference.

[0016] The foregoing is offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that the materials, dimensions, usages, and other parameters of the system described herein may be further modified or substituted in various ways without departing from the spirit and scope of the invention.

Claims

1. In a chromatography column cartridge comprising:

a rigid cylindrical tube defining a separatory resin chamber and a direction of flow of carrier fluid therethrough,
a pair of resin-retaining, liquid-diffusing screens, one said screen bordering said chamber at an upstream end thereof and the other bordering said chamber at a downstream end thereof, and
a pair of end plugs, one seated within each end of said cylindrical tube,

20

25

30

the improvement in which said screen bordering said chamber at said upstream end is a filter of sintered, non-woven stainless steel fibers of graded interstitial passages decreasing in size in said direction of flow.

35

2. A chromatography column in accordance with claim 1 in which said graded interstitial passages decrease to a minimum size that is narrow enough to retain particles 10 microns in diameter.

40

3. A chromatography column in accordance with claim 1 in which said graded interstitial passages decrease to a minimum size that is narrow enough to retain particles 5 microns in diameter.

45

4. A chromatography column in accordance with claim 1 in which said graded interstitial passages decrease to a minimum size that is narrow enough to retain particles 1 microns to 3 microns in diameter.

50

5. A chromatography column in accordance with claim 1 in which said graded interstitial passages decrease to a minimum size that is narrow enough to retain particles 10 microns in diameter, and said

screen has a thickness of from about 0.1 mm to about 1.0 mm and a permeability factor of from about 4×10^{-13} to about $1 \times 10^{-10} \text{ m}^2$.

6. A chromatography column in accordance with claim 1 in which said graded interstitial passages decrease to a minimum size that is narrow enough to retain particles 5 microns in diameter, and said screen has a thickness of from about 0.2 mm to about 0.6 mm and a permeability factor of from about 4×10^{-13} to about $1 \times 10^{-11} \text{ m}^2$.

7. A chromatography column in accordance with claim 1 in which said graded fiber diameter decrease to a minimum size that is narrow enough to retain particles 1 microns to 3 microns in diameter, and said screen has a thickness of from about 0.3 mm to about 0.6 mm and a permeability factor of from about 4×10^{-13} to about $4 \times 10^{-12} \text{ m}^2$.

55

60

65

70

75

80

85

90

95

100

105

110

115

120

125

130

135

140

145

150

155

160

165

170

175

180

185

190

195

200

205

210

215

220

225

230

235

240

245

250

255

260

265

270

275

280

285

290

295

300

305

310

315

320

325

330

335

340

345

350

355

360

365

370

375

380

385

390

395

400

405

410

415

420

425

430

435

440

445

450

455

460

465

470

475

480

485

490

495

500

505

510

515

520

525

530

535

540

545

550

555

560

565

570

575

580

585

590

595

600

605

610

615

620

625

630

635

640

645

650

655

660

665

670

675

680

685

690

695

700

705

710

715

720

725

730

735

740

745

750

755

760

765

770

775

780

785

790

795

800

805

810

815

820

825

830

835

840

845

850

855

860

865

870

875

880

885

890

895

900

905

910

915

920

925

930

935

940

945

950

955

960

965

970

975

980

985

990

995

1000

1005

1010

1015

1020

1025

1030

1035

1040

1045

1050

1055

1060

1065

1070

1075

1080

1085

1090

1095

1095

1100

1105

1110

1115

1120

1125

1130

1135

1140

1145

1150

1155

1160

1165

1170

1175

1180

1185

1190

1195

1200

1205

1210

1215

1220

1225

1230

1235

1240

1245

1250

1255

1260

1265

1270

1275

1280

1285

1290

1295

1300

1305

1310

1315

1320

1325

1330

1335

1340

1345

1350

1355

1360

1365

1370

1375

1380

1385

1390

1395

1400

1405

1410

1415

1420

1425

1430

1435

1440

1445

1450

1455

1460

1465

1470

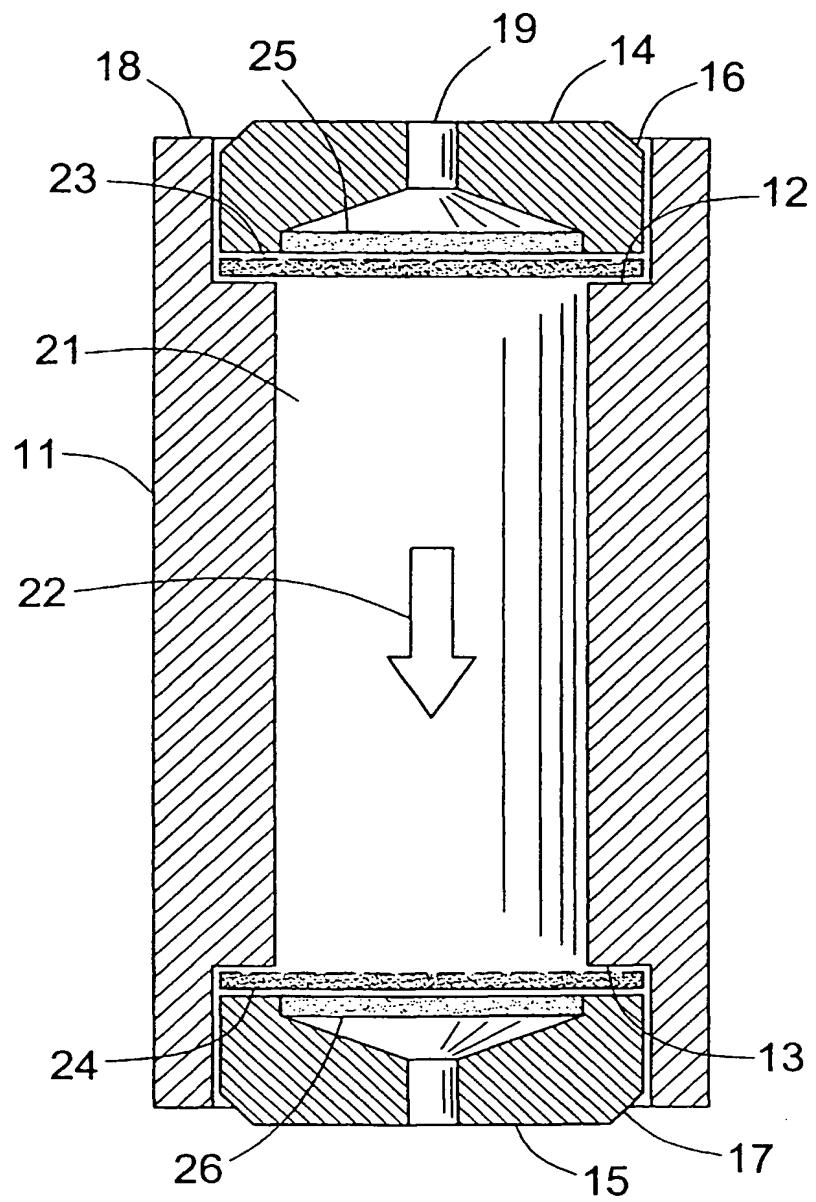
1475

1480

1485

1490

Fig. 1





European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 99 30 6500

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.)
Y	US 5 423 982 A (JUNGBAUER ALOIS ET AL) 13 June 1995 (1995-06-13) * column 4, line 45 - column 5, line 30 * ---	1-7	G01N30/60
Y	US 5 665 479 A (LONCKE JAN ET AL) 9 September 1997 (1997-09-09) * the whole document *	1-7	
A	EP 0 310 867 A (SHIMADZU TECHNO RESEARCH INC) 12 April 1989 (1989-04-12) * page 4, line 1 - page 5, line 43 *	1	
A	PATENT ABSTRACTS OF JAPAN vol. 013, no. 547 (P-971), 7 December 1989 (1989-12-07) & JP 01 227959 A (HITACHI LTD; OTHERS: 01), 12 September 1989 (1989-09-12) * abstract *	1	
	-----		TECHNICAL FIELDS SEARCHED (Int.Cl.)
			G01N
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
MUNICH	25 October 1999	Müller, T	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EP 0 982 587 A1

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 99 30 6500

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

25-10-1999

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5423982	A	13-06-1995	AU	679860 B	10-07-1997
			AU	2650895 A	21-12-1995
			CA	2191464 A	07-12-1995
			EP	0767697 A	16-04-1997
			JP	10501335 T	03-02-1998
			WO	9532782 A	07-12-1995
US 5665479	A	09-09-1997	NONE		
EP 0310867	A	12-04-1989	JP	1094260 A	12-04-1989
			CN	1032457 A	19-04-1989
JP 01227959	A	12-09-1989	NONE		

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

THIS PAGE BLANK (USPTO)